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Hepatic Nervous System in Development, Regeneration, and Disease

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The liver is innervated by autonomic and sensory fibers of the sympathetic and parasympathetic nervous systems that regulate liver function, regeneration, and disease. Although the importance of the hepatic nervous system in maintaining and restoring liver homeostasis is increasingly appreciated, much remains unknown about the specific mechanisms by which hepatic nerves both influence and are influenced by liver diseases. While recent work has begun to illuminate the developmental mechanisms underlying recruitment of nerves to the liver, evolutionary differences contributing to species-specific patterns of hepatic innervation remain elusive. In this review, we summarize current knowledge on the development of the hepatic nervous system and its role in liver regeneration and disease. We also highlight areas in which further investigation would greatly enhance our understanding of the evolution and function of liver innervation. (Hepatology 2021;74:3513-3522).

The liver performs diverse functions to enable life of the organism, including maintenance of metabolic homeostasis, production of blood clotting factors and serum proteins, and detoxification of internal metabolites and chemicals. Autonomic and sensory nerve fibers modulate multiple aspects of liver function, repair, and regeneration, including lipid and glucose metabolism, bile secretion, and cell proliferation after injury.[1-5] For more details on the role of the hepatic nervous system in modulating liver function, we refer readers to several recent reviews.[1-4] Liver disease can be caused by viral and other infections, metabolic disorders, toxin exposure, and genetic diseases. Chronic liver disease and liver failure are responsible for approximately 2 million deaths worldwide annually.[6] Currently, liver transplantation is the only curative treatment for acute liver failure or chronic liver disease. Transplant results in effective liver denervation, which may contribute to higher rates of obesity, hypertension, dyslipidemia, and diabetes in liver transplant patients.[7-12] In contrast, the effects of hereditary, autonomic, and diabetic neuropathies on human liver innervation and function are almost completely unknown. Although the importance of the hepatic nervous system has gained increasing appreciation in recent years, there still remains much to be discovered. Given the fundamental relationship between the nervous system and the liver, a better understanding of the role of hepatic innervation in liver regeneration and dysfunction could lead to new therapeutic approaches for liver diseases. Such efforts would be greatly aided by the development of new genetic tools to allow the specific labeling, isolation, and transcriptional profiling of hepatic neurons. In this review, we discuss the development of liver innervation and the role of the hepatic nervous system in liver regeneration and disease.

Abbreviations: 6-OHDA, 6-hydroxydopamine; CCl4, carbon tetrachloride; CGRP, calcitonin gene-related peptide; HFD, high-fat diet; Ngf, nerve growth factor.

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Anatomy of Hepatic Innervation

The liver is organized into hexagonal functional units termed lobules. Portal triads, located at the corners of the lobules, consist of a bile duct and branches of the hepatic artery and portal vein. Autonomic and sensory nerve fibers enter the mammalian liver at the hilum and form plexuses along the two major blood vessels entering the liver: an anterior plexus surrounding the hepatic artery and a posterior plexus surrounding the portal vein. Additional innervation is found around the hepatic veins.[3,13] Within individual liver lobules, innervation surrounds the hepatic artery, portal vein, and bile ducts (Fig. 1). Preganglionic sympathetic innervation originates from T7-T12, while postganglionic sympathetic innervation from the splanchnic nerve arises in the coeliac and superior mesenteric ganglia.[14,15] Parasympathetic innervation derives from the vagus nerve. In addition to cholinergic and catecholaminergic neurotransmitters, hepatic neurons produce several neuropeptides, including neuropeptide Y, substance P, vasoactive intestinal peptide, calcitonin-gene related peptide, glucagon-like peptide, somatostatin, neurotensin, and serotonin.[16-18] Expression of α-adrenergic and β-adrenergic receptors, as well as muscarinic and nicotinic cholinergic receptors, has been identified in multiple liver cell types. For example, cholangiocytes express both α1-adrenergic and β1-adrenergic receptors, as well as M3 cholinergic receptors.[19,20]

Although the portal tract is innervated in most species examined, the distribution of nerves within the liver is highly species-specific (Table 1). Electron microscopy studies of monkey, rabbit, guinea pig, rat, golden hamster, pigeon, quail, and turtle livers demonstrated that innervation surrounds the hepatic artery, bile duct, and portal vein in all these species.[21] Nerve fibers were also found in direct contact with hepatocytes, with the exception of rat and golden hamster.[21,22] In all species, parasympathetic innervation appears restricted to the portal triad; when present, parenchymal innervation is sympathetic.[23] In contrast to mammals, birds and reptiles, innervation of the frog liver is limited to large blood vessels. Although nerve fibers are not apparent in the newt or loach liver,[21] both blood vessels and bile ducts are innervated in the rainbow trout liver.[24] To better understand the evolution of liver innervation, it will be informative to examine a wider array of amphibian and fish species across the evolutionary spectrum. Additional data from genetically tractable species such as zebrafish, which have already been leveraged to study liver development and disease, would be particularly useful.

Development of the Hepatic Nervous System

Although significant effort has been devoted to studying the anatomy and function of hepatic innervation across species, our understanding of the ontogeny of liver innervation is still developing. In humans, nerve fibers are first seen at the hilum at 8 weeks, while portal tract innervation can first be detected starting at week 12. Intrasinusoidal fibers do not appear until week 28.[25] In mice, innervation is initially observed at extrahepatic bile ducts at E17.5, and fibers extend into the liver throughout the first postnatal weeks.[26,27] Murine biliary epithelial cells and neighboring mesenchymal cells express nerve growth

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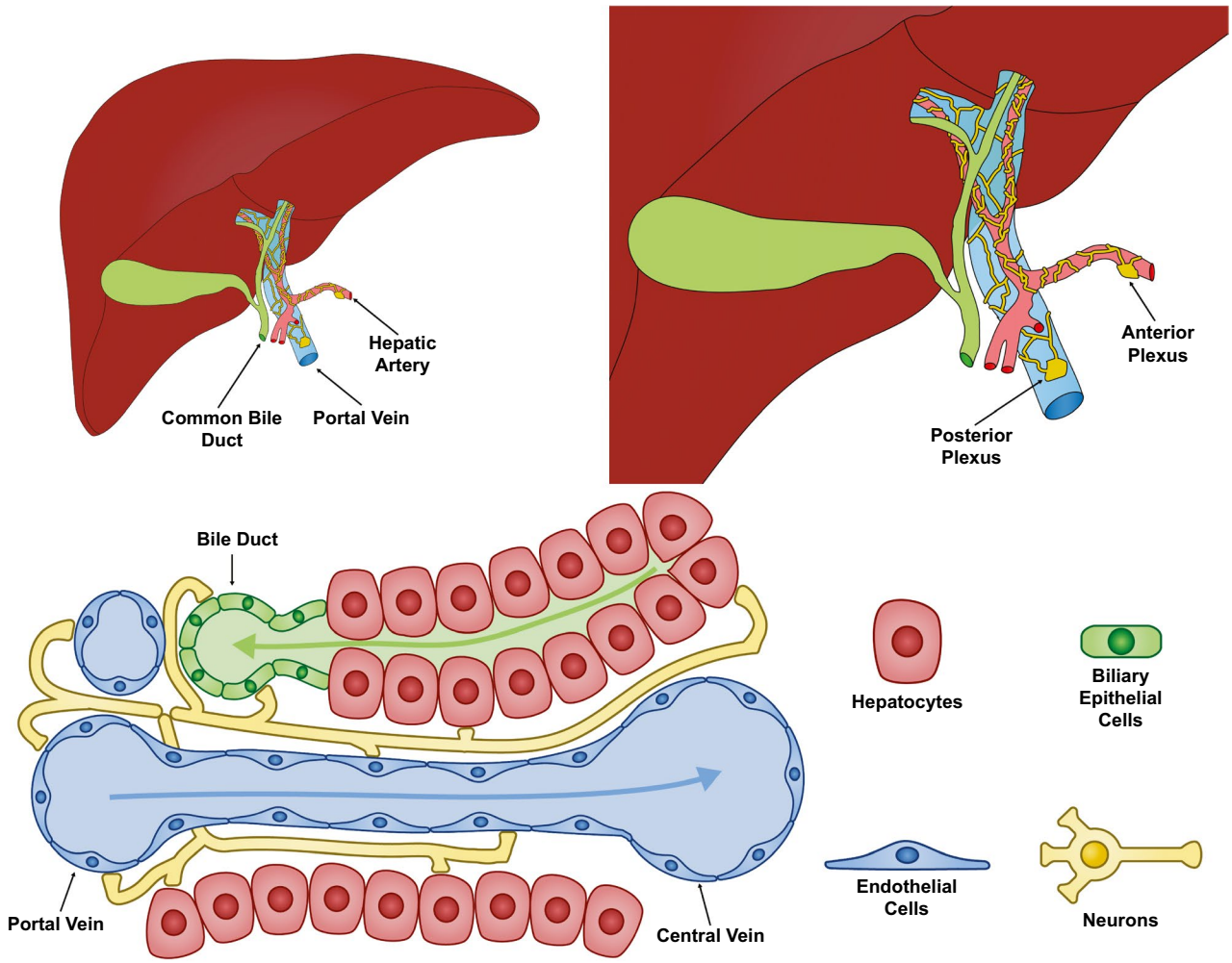
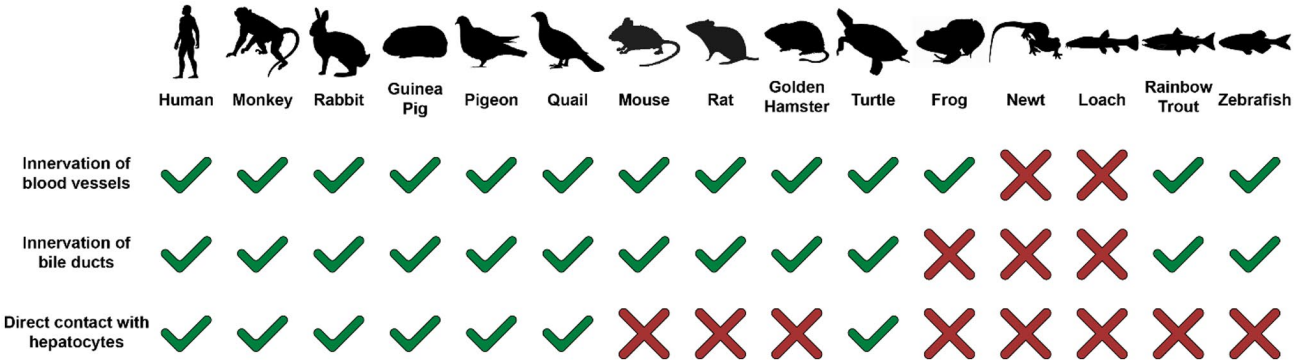


FIG. 1. Innervation of the human liver. Nerves enter the liver at the hilum, forming an anterior plexus around the hepatic artery and a posterior plexus around the portal vein. Within the liver, nerves are found around the hepatic artery, portal vein, and bile ducts. Sympathetic nerves, but not parasympathetic nerves, synapse on hepatocytes as well. Innervation around hepatic veins is generally sparse when present.

factor (*Ngf*), and ectopic expression of *Ngf* in hepatocytes is sufficient to induce extension of nerve fibers from the periportal to the pericentral area.[27] Loss of *Ngf* has also been reported to greatly reduce the extent of liver innervation in mice[28]; however, livers were assessed at E16.5 and P0.5, when most of the hepatic nerve network has not yet been established.

Many open questions surrounding the development of hepatic innervation remain. Further comparative work examining the mechanisms governing development of hepatic innervation across species would illuminate potential developmental bases for evolutionary elaboration of the hepatic nerve network, while ascertaining patterns of liver innervation in patients with disorders of bile duct formation would indicate whether biliary cells also drive hepatic innervation in humans. Whether autonomic input regulates liver development is also unclear. Given the relatively late developmental timing of liver innervation, it seems reasonable to hypothesize that a role for autonomic innervation in liver development might be restricted to maturation or refinement of liver function. A better understanding of the development of hepatic innervation would permit comparisons between mechanisms of innervation and reinnervation in liver development, injury, and regeneration.

TABLE 1. Distribution of hepatic neurons varies across species. All mammals studied, as well as birds and reptiles, have innervation surrounding the blood vessels and bile ducts, but nerves do not always synapse directly with hepatocytes.[21,27] Innervation of hepatocytes is not seen in amphibian and fish species that have been studied to date, and some of these species lack intrahepatic innervation altogether[21,24] (zebrafish data: Miller and Goessling, unpublished).



The Hepatic Nervous System in Liver Repair and Regeneration

The liver is capable of compensatory regeneration after toxic or physical injury via a combination of cellular hypertrophy and hyperplasia.[29,30] Experimental liver injury models highlight the role of sympathetic and parasympathetic nerves in liver repair; however, how different injury modalities prompt varying responses from the hepatic nervous system is not entirely clear. Here, we focus on the most well-characterized models from an autonomic nervous system perspective: partial hepatectomy and toxic injury from carbon tetrachloride (CCl_4) or acetaminophen exposure.

PARTIAL HEPATECTOMY

Liver tissue mass is restored following partial hepatectomy by proliferation and hypertrophy of hepatocytes in the remaining lobe.[29,30] Hepatic vagotomy negatively affects the proliferative response to partial hepatectomy, whereas sympathetic nervous system activity may be suppressed during early stages of regeneration.[31-34] In rats, hepatic vagotomy results in significantly smaller liver-to–body weight ratios 7 days after partial hepatectomy due to decreased hepatocyte proliferation.[32] Hepatic vagotomy before partial hepatectomy results in increased mortality in mice, an effect reversed by overexpression of *FoxM1*, which regulates hepatocyte proliferation as part of a vagus-macrophage-hepatocyte signaling network. Following partial hepatectomy, vagus-derived cholinergic signals prompt IL-6 release from macrophages and result in increased hepatocyte proliferation in a FoxM1-dependent manner.[35] In contrast, sympathetic denervation of the liver has no effect on liver-to–body weight ratio after partial hepatectomy.[32]

Sensory nerves also promote hepatocyte proliferation following partial hepatectomy. Calcitonin gene-related peptide ($CGRP^+$) neurons innervate the biliary tree, and *CGRP* expression increases in rat livers following partial hepatectomy.[36] RAMP1 is a component of the CGRP receptor, and *RAMP1* knockout mice show decreased hepatocyte proliferation and significantly delayed regeneration following partial hepatectomy.[37]

TOXIC INJURY MODELS

Data from toxic injury models indicate that the sympathetic nervous system exerts a pro-inflammatory, profibrotic effect by acting on hepatic stellate cells (HSCs). Sympathetic nerves are in close proximity to HSCs, and activated HSCs express α-adrenergic and β-adrenergic receptors, as well as receptors for neuropeptide Y.[38,39] Catecholamines induce HSC proliferation and extracellular matrix protein production *in vitro*.[39-41] Dopamine-β-hydroxylase knockout mice cannot produce norepinephrine and have reduced hepatic α–smooth muscle actin accumulation when fed an antioxidant-deficient diet that produces fibrosis in control mice.[42]

CCl_4 toxicity induces hepatocellular necrosis and steatosis, as well as inflammation. Ablation of sympathetic nerves with 6-hydroxydopamine (6-OHDA) before CCl_4 treatment reduces necrosis, steatosis, and expression of pro-inflammatory cytokines in mice.[43] 6-OHDA is a synthetic catecholaminergic neurotoxin that is taken up by the dopamine active transporter and induces neuronal apoptosis through production of reactive oxygen species and inhibition of complex I of the mitochondrial respiratory transport chain.[44] Spontaneously hypertensive rats have increased peripheral sympathetic activity[45] and are sensitized to CCl_4-induced liver toxicity, developing cirrhosis after 4 weeks of CCl_4 administration, while control rats display a less severe phenotype of bridging fibrosis.[46] Hepatocytes damaged by CCl_4 exposure undergo apoptosis, and sympathetic denervation increases hepatocyte apoptosis and decreases hepatocyte proliferation.[47] Prevention of apoptosis by sympathetic signaling could result in accumulation of damaged cells that produce a persistent inflammatory response and increased fibrosis.[47] Accordingly, β-receptor agonists and α1-receptor antagonists also have protective effects in acetaminophen-induced liver injury.[48,49]

In contrast, parasympathetic neurons exert a protective effect on hepatic inflammation by acting through α7nAChR expressed on macrophages. In anti-Fas-treated mice, hepatic vagotomy significantly increases mortality, but co-treatment with an α7nAChR agonist attenuates this effect.[50] Vagal signaling also decreases inflammation in lipopolysaccharide-induced hepatitis, likely through macrophage-expressed α7nAChR.[51] In murine acetaminophen-induced liver injury, acetylcholinesterase inhibitors improved survival and reduced hepatocellular damage, apoptosis, and inflammation.[52] Therefore, in multiple models of chemical liver injury, vagal signaling has a protective, anti-inflammatory role. Sympathetic neurons, in contrast, promote inflammation and liver damage in most hepatocyte injury models.

BILIARY INJURY

Neural regulation of cholangiocytes is often assessed through bile duct ligation in rats, a procedure that prompts cholangiocyte proliferation and liver fibrosis. 6-OHDA-induced sympathetic denervation of bile duct–ligated rats reduces proliferation and increases apoptosis of cholangiocytes.[53] Similarly, both α-CGRP knockout and hepatic vagotomy decrease cholangiocyte proliferation and increase apoptosis after bile duct ligation.[20,54] Therefore, sympathetic, parasympathetic, and sensory nerves perform either redundant or synergistic functions in promoting cholangiocyte proliferation and reducing apoptosis following bile duct injury.

EFFECT OF EXPERIMENTAL LIVER INJURY ON HEPATIC INNERVATION

Hepatic injury results in dynamic changes in nerve distribution within the liver. However, the mechanisms governing alterations in hepatic innervation in different injury contexts are largely unknown, as are the consequences of altered innervation for liver function and pathology. In rats, partial hepatectomy precipitates an immediate decrease in portal tract innervation in the unresected tissue. Innervation density in the remaining liver lobe then returns to control levels over 6 months, as lost tissue mass is replaced.[55-57] In contrast to portal tracts, hyperinnervation is seen at the hepatic hilum 1 week after partial hepatectomy.[57] Innervation of regenerating hepatic tissue is thought to derive from remaining axons in the liver rather than from ingrowth of new axons.[57] Acute CCl_4 injury is followed by an immediate decrease in noradrenergic staining in the liver, whereas chronic CCl_4 injury leads to the appearance of nerve terminals in fibrous septa in close contact with mast cells and fibroblasts.[58-60] Ligation of the common bile duct in guinea pigs produces a complete loss of portal tract innervation, with nerves remaining only at the hilum.[61] Nerves were also later present in connective tissue of fibrotic areas.[61,62]

Hepatic Innervation in Liver Diseases

Central and peripheral nervous system dysfunction are common side effects of liver disease.[63-65] Emerging evidence also points to a role for the peripheral nervous system in the development and progression of liver diseases such as NAFLD and HCC.

NAFLD

Metabolic syndrome is a major risk factor for NAFLD, with studies suggesting that NAFLD is the hepatic manifestation of metabolic syndrome.[66,67] Increased sympathetic activity is predictive for the development of metabolic abnormalities, and decreased activity of the parasympathetic nervous system is also associated with metabolic syndrome.[68,69] Patients with NAFLD have higher odds of displaying autonomic dysfunction,[70,71] raising the question of whether autonomic dysfunction early in the disease could enhance development and progression of NAFLD. Indeed, mice fed a high-fat diet (HFD) develop hepatic sympathetic overactivity.[72] Sympathetic denervation reduces HFD-induced steatosis and hepatomegaly, indicating that sympathetic nervous system overactivity directly contributes to the development of diet-induced steatosis.[72] Another recent study demonstrates that serotonin released in response to autonomic liver-gut signaling may contribute to NAFLD.[73] HFD has also been shown to induce reversible hepatic sympathetic neuropathy in mice as a result of elevated macrophage-derived TNF-α signaling through *Sarm1* (sterile alpha and TIR motif containing 1) expressed on sympathetic neurons.[74] Therefore, metabolic stress affects both survival and function of hepatic sympathetic neurons.

Maternal HFD produces NAFLD-associated changes in fetal macaque livers, including increased hepatic triglycerides and oxidative stress.[75] Perinatal exposure to HFD has also been found to reduce sympathetic innervation and increase hepatocyte apoptosis in male macaques.[76] These findings indicate that some metabolic diseases in adulthood may have their origin not just in developmental disturbances, but in specific effects on sympathetic innervation that develops during the fetal period.

While overactivity of the sympathetic nervous system can contribute to development of NAFLD, parasympathetic activity may play a protective role by dampening HFD-driven inflammation. Treatment of HFD-fed mice with galantamine, which increases cholinergic signaling, significantly improved hepatic steatosis.[77] In methionine-deficient, choline-deficient diet-induced steatohepatitis, hepatic vagotomy promotes liver inflammation and macrophage activation.[78] In summary, current evidence demonstrates that HFD can produce imbalances in autonomic nervous system activity; conversely, autonomic nervous system dysfunction can directly contribute to the development of NAFLD.

HCC

The autonomic nervous system is increasingly recognized as a significant contributor to tumor progression and metastasis.[79,80] The most common type of liver cancer is HCC, an aggressive cancer that is often diagnosed late in the disease course when effective treatment options are limited. In HCC hepatectomy samples, high sympathetic fiber density is associated with poor prognosis.[81] Expression of monoamine oxidase A (MAOA), an enzyme involved in catecholamine neurotransmitter breakdown, is down-regulated in HCC, and higher levels of MAOA are associated with both improved survival and disease-free survival.[82] In DEN-induced HCC in rats, sympathetic denervation through 6-OHDA reduced fibrosis and tumor formation.[81]

IMPACT OF LIVER DISEASE ON HEPATIC INNERVATION

Many liver diseases are associated with altered patterns of hepatic innervation. Increased portal vein innervation is seen in noncirrhotic portal hypertension, alcoholic hepatitis, and toxic liver injury.[83,84] In contrast, regenerating nodules in cirrhotic livers of multiple etiologies lack parenchymal innervation, but retain innervation in portal tracts and fibrous septa separating regenerative nodules.[83,85-87] Parenchymal nerve fiber density is reduced but not absent in precirrhotic livers, suggesting that loss of parenchymal nerve fibers is a consequence of worsening disease course.[86] Whether nerve degeneration contributes to disease progression is unclear, as are the mechanisms underlying loss of parenchymal fibers.

Biliary tree innervation is also altered in several biliary diseases. Extrahepatic biliary obstruction and hepatolithiasis are associated with increased innervation of intrahepatic bile ducts.[88] In contrast, patients with biliary atresia lack intrahepatic bile duct innervation but show increased hepatic artery and portal vein innervation.[89] Given that intrahepatic bile ducts guide liver innervation in mice, it is tempting to speculate that reduced bile duct innervation in biliary atresia may be a developmental phenotype secondary to

aberrant gene-expression programs in biliary cells. An alternative possibility is that biliary innervation forms normally and then degrades.

LIVER TRANSPLANT AND ACUTE SPINAL CORD INJURY

The extent of reinnervation following liver transplant is unclear. While intralobular innervation is not re-established, reinnervation of portal tracts has been observed.[7-9,90-92] Transplanted livers have reduced norepinephrine and epinephrine content compared with controls, suggesting that sympathetic activity is nevertheless decreased.[7] In contrast to liver transplant, acute spinal cord injury results in sympathetic nerve overactivity.[93-98] Comparing outcomes following liver transplant and spinal cord injury presents a unique opportunity to assess the role of hepatic nervous system activity in human liver function and pathology.

Transplant-induced denervation has well-defined effects on graft function in several systems.[99-101] The effects of denervation on the transplanted liver are less clear, but several alterations in liver function have been noted. Compared with nontransplant patients on immunosuppressive medication, liver transplant patients have elevated insulin and glucagon and defective counter regulation following hypoglycemia.[102] Liver transplant also results in increased postprandial hyperglycemia compared with kidney transplant.[103] Hepatic vagotomy reduced cholangiocyte proliferation and increased apoptosis in common bile duct–ligated rats.[20] In agreement with these experimental data, transplanted livers with hepatitis have fewer proliferative ductal cells than livers of kidney transplant patients with hepatitis.[54] These studies suggest that hepatic denervation could contribute to increased rates of metabolic syndrome in liver transplant recipients.[104] However, immunosuppressive medication also increases the risk of diabetes, hyperlipidemia and hypertension,[104] so further studies directly comparing metabolic alterations in liver transplant patients to other organ transplant patients will be helpful in disentangling these factors.

Spinal cord injury at cervical or thoracic levels precipitates both acute and chronic liver pathology. Following spinal cord injury, an immediate systemic inflammatory response amplified by factors from the liver is initiated.[105-107] Chronic inflammation then persists in the liver for up to 6 months in rodent models.[108] The acute phase of injury is also characterized by liver fat accumulation,[108-110] and steatosis persists chronically and is present in 20%-50% of patients with spinal cord injury assessed by ultrasound.[111,112] The sympathetic nervous system regulates hepatic *de novo* lipogenesis, fatty acid uptake, and lipolysis.[2] Increased activity of the sympathetic nervous system, as seen in spinal cord injury patients, drives both inflammation following injury and the development of steatosis in experimental models.[43,46,47,72,81] Therefore, sympathetic overactivity in patients with spinal cord injury may be a driving factor for high rates of NASH in this population.

Conclusion

The hepatic nervous system regulates liver function, regeneration, and disease. Dysregulation of hepatic autonomic inputs contributes to the development of liver pathology, particularly steatosis and inflammation, and liver disease can in turn produce autonomic dysfunction, which raises the possibility that autonomic dysfunction arising as a result of chronic liver diseases could then exacerbate those liver diseases. Multiple liver diseases and experimental liver injury models are associated with dynamic alterations in patterns of liver innervation, but the mechanisms underlying these observations remain to be elucidated. The specific hepatic structures receiving innervation vary among species, especially when comparing fish and amphibians to mammals, birds, and reptiles. How differences in developmental programs guiding hepatic innervation contribute to this evolutionary variation in hepatic innervation is not well understood. Further studies in these areas will be useful both to develop therapeutics for diseases such as NAFLD that have demonstrated autonomic involvement and to better understand how disease-associated changes in hepatic innervation affect liver function and pathology going forward.

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I.M.O. contributed to the visualization. B.M.M., I.M.O., and W.G. contributed to the reviewing and editing. W.G. supervised the study and acquired funding.

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